

Original Research Article

Inducible and Constitutive Clindamycin Resistance in *Staphylococcus* spp. Strains Isolated from a Neonatal Intensive Care Unit

ABSTRACT

Aims: The environment can act as a reservoir for microorganisms, which in turn can contaminate a range of hospital equipment and survive for long periods of time. One of these environments is the Neonatal Intensive Care Unit (NICU), home newborns with low weight and needing invasive procedures for administration of nutritional and medicinal substances, which makes the NICU a critical area for housing individuals with immune system immature. Thus, the aim of this study was to evaluate the phenotypic appearance of resistance of *Staphylococcus* spp. isolated areas in a NICU in the city of Campina Grande - PB, compared to erythromycin and clindamycin. Samples were collected from surfaces present at the NICU.

Place and Duration of Study: Sample: Neonatal Intensive Care Unit of a public hospital in the city of Campina Grande – PB, Brazil. Processing and Analysis of Samples: Clinical Analysis Laboratory of the State University of Paraíba, between August and October 2012.

Methodology: Samples were collected from surfaces present at the NICU. The same were you identify and strains of *Staphylococcus* spp. were subjected to sensitivity, and to verify erythromycin-induced resistance, we used D-test, following the CLSI standards-M100-S22 (2012).

Results: Bacterial strains from all surfaces analyzed were isolated, 59.02% of isolated batteries belong to the genus *Staphylococcus* spp., Representing 36 bacterial strains, of which 31 species *Staphylococcus aureus* out of 5 and *Staphylococcus* spp., There was a fee more than 70% resistance to the group of penicillins, more than 30% methicillin. Among the 36 strains of *Staphylococcus* spp. 19.45% were resistant to erythromycin. The rate found for constitutive resistance to macrolides (MLSBc) was 5.56% and induced resistance to the macrolide type (MLSBi) 2.78% was observed in the strains.

Conclusion: Paramount importance periodic resistotyping with antibiotics selected by competent bodies, whose resistances are important genetic and epidemiological markers in order to distinguish new lines *Staphylococcus* spp., those endemic, as well as a better understanding of the evolution of drug resistance in a unique environment.

Keywords: *Staphylococcus* spp, contaminated surfaces, bacterial resistance, MLSB.

1. INTRODUCTION

In recent decades, with advances in staphylococcus classification and the development of new gender identification techniques, species and subspecies, there have allowed researchers and clinicians become more aware of the wide variety of coagulase-negative staphylococcus (CNS), the which were previously described as non-pathogenic, are now recognized as opportunistic microorganisms that prevail in many different situations to produce serious infections [1]. The transmission of these micro-organisms can occur by direct contact, where health professionals to come into contact with patients colonized or objects, can serve as transmitters of organisms to other patients. Not to mention the

23 environmental and airway transmissions, which are uncommon in certain cases, but in some
24 circumstances may occur [2]).

25 So even though the exact function in the inanimate environment plays staphylococcus
26 transmission is not yet determined, it can act as a reservoir for microorganisms, which in turn
27 can contaminate a range of hospital equipment and survive for long periods of time [3,4,5].
28 One of these environments it is the neonatal ICU, home newborns with low birth weight, and
29 immunologically immature that require invasive procedures for administration of nutritional
30 and medicinal substances [1]. Thus, due to the immaturity of the immune system of the
31 newborn and the use of broad spectrum antibiotics, species commonly found in the air can
32 become pathogens, making the units of pediatric intensive care and neonatal critical areas
33 [6,7,8].

34 In addition to that, a growing number of micro-organisms develop resistance to drugs used
35 to treat infections, as they are also impervious to new drugs [9]. Among them, has been
36 isolated in recent years coagulase negative Staphylococcus (CNS) resistant to multiple
37 antimicrobials, which has leveraged the interest in studying their susceptibility commercial
38 drugs [10]. Given that studies have shown multiresistant microorganisms to antimicrobial
39 surfaces of beds and equipment after cleaning and disinfection inappropriate, interest in this
40 monitoring is only going to increase further [11,12]

41 Recently, the detection of macrolide-lincosamide-streptogramin resistance (MLS) between
42 Staphylococcus has attracted the attention of clinical laboratories. Three mechanisms have
43 been reported to the MLS B resistance to antibiotics: modification of the site of action, efflux
44 of antibiotics and modifying drugs [13]. MLSB carries the erm gene (erythromycin ribosome
45 methylase) encoding rRNA methylase which modifies the binding site of the antimicrobial
46 agent by mutation or methylation of 23S rRNA, resulting in resistance to macrolides,
47 lincosamides, and streptogramin B. There are four main classes erm gene [erm (A), erm (B),
48 erm (C) and erm (C)], and the types erm (A) and erm (C) often responsible for resistance in
49 staphylococci MLSB [14,13].

50 The strains that express the MLSB phenotype can be classified as induced (MLSBi) or
51 constitutive (MLSBc). When an MLSB inducible strain is exposed to an inducer (such as a
52 low-erythromycin), increases the level of expression of rRNA methylase resulting in an
53 increased resistance MLS class of antimicrobials (such as clindamycin). Even if the strains
54 with an inducible erm gene are resistant to inducers and do not remain susceptible to
55 macrolides and lincosamides inductors, in general, different inducible MLSB resistance
56 patterns can be observed according to the type of erm gene or its expression level. In this
57 case, it is necessary to perform disk-approximation induction test (D-test). In constitutive
58 resistance methylase mRNA produced is active even in the absence of an inducer, which
59 gives a characteristics, a high level of cross-resistance to drugs of MLS B group [13,15,16].

60 The msrA gene is responsible for the efflux mechanism for staphylococci, which is activated
61 after exposure to a macrolide where pumps 14 or 15 members macrolides and
62 streptogramin type B [17, 13]. Therefore, the bacteria are resistant to erythromycin but
63 remained susceptible to clindamycin, since it is not an inducer and a substrate for the pump.
64 When the strains, which are previously sensitive to streptogramin B, become resistant after
65 exposure of a macrolide phenotype are classified as type M or efflux MSB and can be
66 differentiated from MLSBi phenotype by using double-disk testing, showing no interactions
67 between erythromycin and clindamycin in this case (no D-shaped zone). Resistance to
68 macrolides mediated efflux is common among coagulase-negative staphylococci (CNS) and
69 it is increasingly found in methicillin-sensitive Staphylococcus aureus (MSSA) [18].

Some researchers recommend that treatment with clindamycin is avoided in cases of infections caused by *S. aureus* strains that exhibit MLSBi phenotype, since there are reports of clinical failures in the treatment of patients with this phenotype resistance [19,20,21,22]. Similarly, the classification of all *S. aureus* resistant to erythromycin and clindamycin-resistant can prevent the clindamycin is used in cases where the drug would be an effective alternative treatment [23]. The presence of these drug-resistant strains is very serious, as the antimicrobial of this group are therapeutic options for treatment of staphylococcal infections in skin and soft tissue, and are alternatives to individuals with hypersensitivity to penicillin [24]. Thus, the characterization of the sensitivity profile and the investigation of microorganisms with MLSBi resistance phenotype are necessary.

Given the above, this study aimed to isolate, identify and assess the sensitivity of *Staphylococcus* spp isolated in cultures of various sites of surfaces in a neonatal intensive care unit in the city of Campina Grande, and assess the phenotypic appearance of resistance of these micro front-organisms to erythromycin and clindamycin, correlating the results with the presence of *ermA* and *msrA* genes.

2. MATERIAL AND METHODS

2.1 Study Design

This is a cross-experimental study in a hospital in the city of Campina Grande, Paraíba, Brazil, in the sector of the Neonatal Intensive Care Unit (NICU). Samples were taken from existing surfaces in the environment: mattress incubators (n = 10), the stethoscope diaphragm (n = 09), door handles (n = 8), taps (n = 4), telephone (n = 1), computer keyboard (n = 1), mouse (n = 1) and monitor (n = 1). These locations were selected due to the fact they are often manipulated, acting as potential sources of contamination by exposure to health professionals. The collection occurred during the months of August and October 2012, with the knowledge and authorization of the hospital board and the head of the hospital's nursing sector under study.

2.2 Sampling

For sample collection, we used a sterile swab moistened with saline 0.9%, where it was rubbed and rolled in random locations of the surfaces of the materials investigated. After collection, the swabs were placed in test tubes containing 5 mL of 0.9% saline, and immediately transferred to a cooler and brought to the Clinical Analysis Laboratory of the State University of Paraíba for sample processing within two hours after collection.

2.3 Isolation and Identification

A 50 ul aliquot was removed 5.0 mL of 0.9% saline solution containing the samples to be analyzed, and they were seeded at spreading plate method on the surface of the culture media blood agar and Mannitol Salt through the uniform spread with a sterile glass rod (Drigalski spatula). Plates were incubated in the greenhouse for 24 - 48 hours at 35⁰-37⁰C. The identification was carried out according to the macroscopic aspects (characteristics of the bacterial colony) and microscopic (Gram stain) of colonies isolated in the culture media used. The bacteria in the Gram staining were identified as Gram-positive cocci, catalase test passed through, where the catalase obtained a result that - positive, were subjected to the latex agglutination assay, based on the search protein clumping factor A and (Staphclin látex®), deoxyribonuclease search using the DNase agar and determining the enzyme activity pyrrolidonil arylamidase - PYR.

118 2.4 Resistance Profile and Resistance to Macrolides

119 The resistance profile was determined by the diffusion with disks method. A bacterial
 120 suspension using 0.9% saline solution was taken and normalized to a concentration in the
 121 range of 0.5 McFarland (1.5×10^8 CFU / ml). Then, the disk containing ampicillin (10mg),
 122 amoxicillin / clavulanic acid (20 / 10mg), ampicillin / subactam (10 / 10mg), cefepime (30µg),
 123 30µg ceftazidime, cefuroxime (30µg), cephalothin (30µg), imipenem (10mg), meropenem
 124 (10mg), gentamicin (10mg), amikacin (30µg), tobramycin (10mg), azithromycin (15µg),
 125 erythromycin (15µg), tetracycline (30µg), cirpofloxacin (5µg), nitrofurantoin (300µg),
 126 clindamycin (2mg), sulfamotexazol / trimethoprim (1.25 / 23.75µg), trimethoprim (5µg),
 127 chloramphenicol (30µg), rifampicin (5µg), linezolid (30µg), penicillin G (10mg), oxacillin
 128 (1mg) and cefoxitin (30µg) (Cecon Ltda. Sao Paulo, Brazil) were placed on plates containing
 129 Mueller Hinton Agar (Difco, Sparks, MD, USA) and then incubated at 37 ° C for 24 to 48
 130 hours. Samples with phenotypic profile of sensitivity clindamycin and erythromycin
 131 resistance exhibited by the agar diffusion test were submitted to the test "D" or the double
 132 disk diffusion test. The test "D" was performed according to CLSI methodology - M100-S22
 133 (2012). After standardization of the sample concentration according to 0.5 MacFarland scale,
 134 the samples were individually grown on Mueller Hinton Agar (Difco, Sparks, MD, USA) and
 135 clindamycin disks (2mg) and erythromycin (15µg) prepared adjacent from 15 to 26 mm from
 136 each other and the plates incubated at 35 ° C for 24 to 48 hours. Induced resistance to
 137 clindamycin was detected by the appearance of the inhibition zone on the "D".

138 3. RESULTS AND DISCUSSION

141 After processing and analysis of samples, were isolated 36 strains of *Staphylococcus* spp.,
 142 Of which 31 were identified as *Staphylococcus aureus* and 5 identified as *Staphylococcus*
 143 spp, as can be seen in Figure 1. According to the literature, a high contamination in the
 144 environment can be a mirror of a bad adherence to hygiene measures both hands as well as
 145 the environment [25]. Research on the occurrence of multiresistant bacteria in an intensive
 146 care unit found that after identification, 55.7% of the identified bacterial strains were
 147 *Staphylococcus* spp., which is in line with the 59.02% found in this work [9].

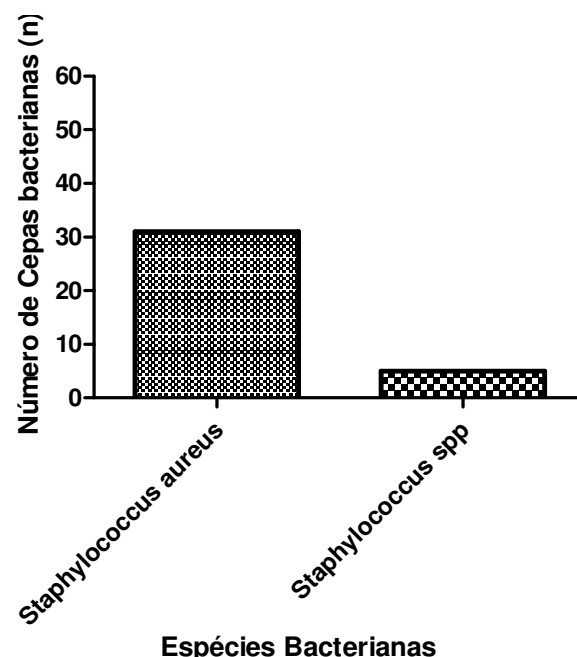


Fig. 1. Proportion of Staphylococcus aureus and Staphylococcus spp. isolated from the analyzed surfaces.

The cultures of the areas analyzed the presence of Staphylococcus spp. 88.89% of the stethoscope 25% of taps, 25% of the handles and 50% of the mattresses. Also isolated the same bacterial genus in single samples analyzed phone, mouse, keyboard and monitor. Figure 2 shows the distribution of the number of isolates in each fomite type. It is observed that the stethoscopes had greater relevance in the number of strains, followed by telephone and mattress.

The surfaces of stethoscopes are a place of proven contamination, but at the same, is not given due importance. [26] The 88.89% infection rate found in this study correlates with values reported by other authors, which evaluated the bacterial contamination rate of stethoscopes and found the value of 86.8 and 97.9% respectively of contamination of equipment [27,26]. According to a previous study, the mattress is one of the objects that have the most contact time with patients, which may serve as a reservoir for infection-causing microorganisms. The same paper cites a percentage of 72.2% of contaminated mattresses. This result proves higher than the 50% observed in this study [25]. Very touched surfaces such as doorknobs, telephones, computers, support the hypothesis that the more you handle such fomites, more contaminated they become. So when professional touch these places, not cling the importance of hand hygiene and can spread microorganisms to other locations or patients [28,25].

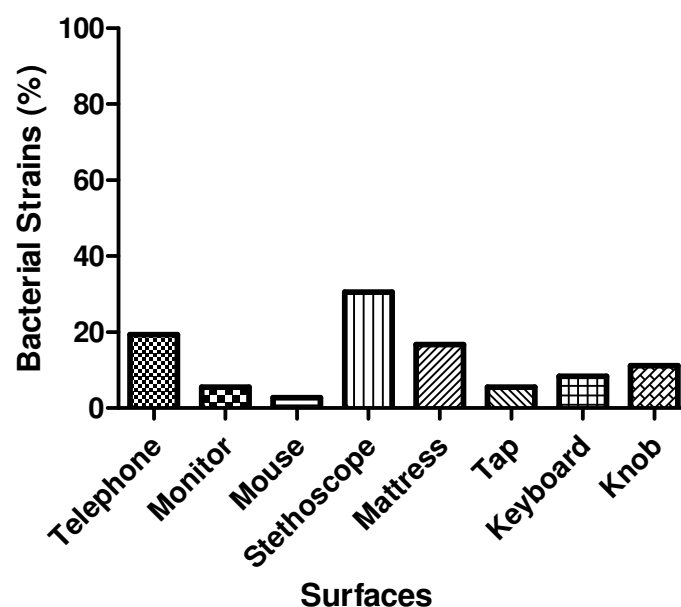
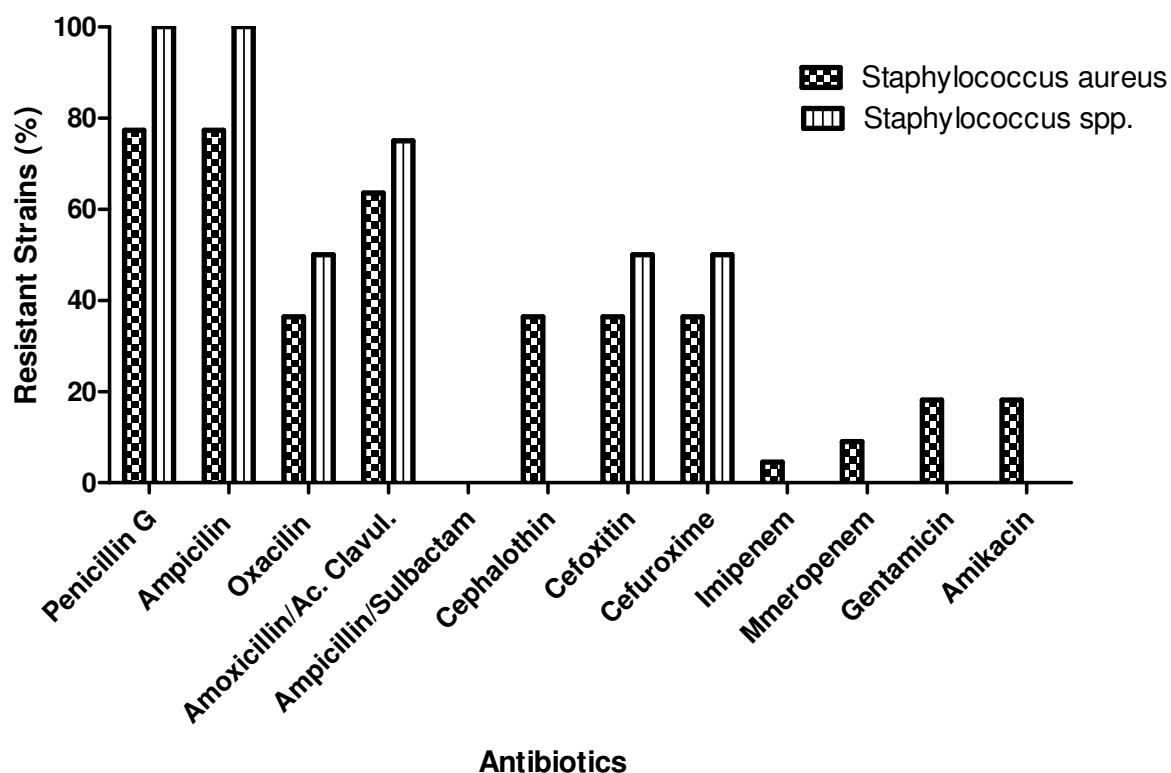


Fig. 2. Distribution of bacterial strains according examined surface.

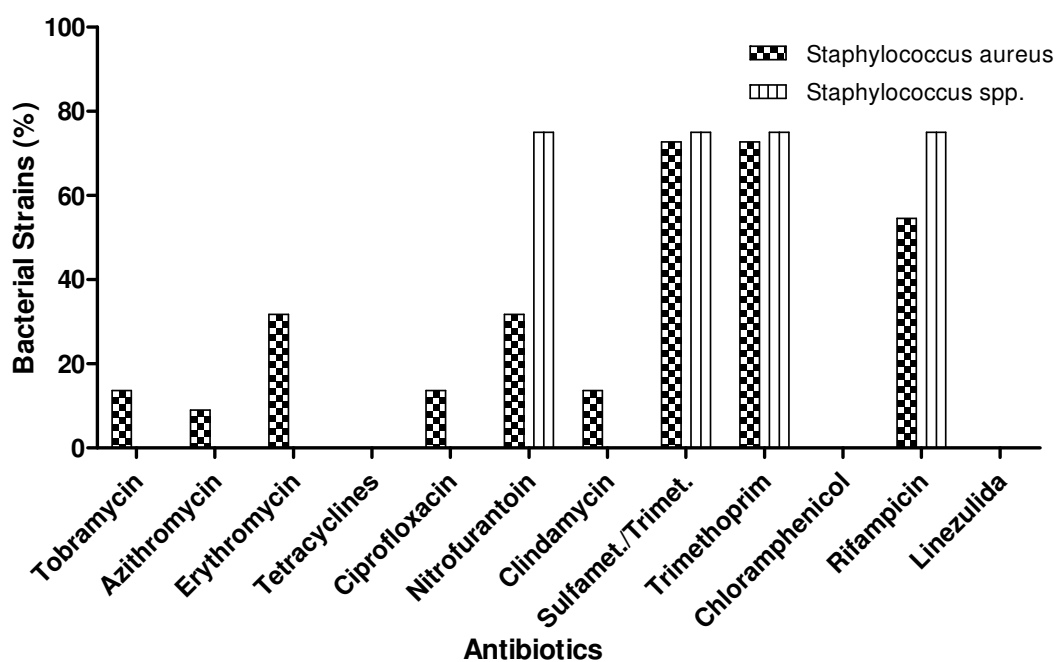
Of the 36 isolates sample, only 26 were resistance profile to all antibiotics mentioned above, the remaining need repeated to confirm the results, which was not possible. Thus, the 26 samples 22 were *Staphylococcus aureus* and 4 *Staphylococcus* spp. In Figures 3 and 4 are the results of antibiotic resistance in the 22 tested strains of *Staphylococcus aureus* and 4 strains of the *Staphylococcus* spp. It is possible to observe a resistance of more than 70% to the class of penicillins. Obtained as a resistance percentage of more than 30% cefoxitin, therefore, this result was extended to cephalothin, cefuroxime and oxacillin, where, in the latter drug, cefoxitin functions as resistance marker. Strains of *S. aureus* showed a resistance percentage of 31.8% of erythromycin, in contrast to the percentage strength of the 9.0% azithromycin antibiotic belonging to the same group and may have extended our results without the need for test the two drugs. Comparing the strains of *S. aureus* and *Staphylococcus* spp., one realizes that *S. aureus* showed a wider resistance profile that *Staphylococcus* spp.

Research conducted in Brazil say that more than 70% of the bacterial strains are isolated, both in community and hospital settings, are resistant to the class of penicillins [29]. This confirms the proportion of 77.3% to penicillin G and ampicillin. Such resistance is already expected, due to the fact that these antibiotics have their widespread use in the treatment of infections, which, at present, the use of these chemotherapy is limited [30]. Such resistance is conferred by the action of penicillinase enzyme, soon after the appearance of penicillins, appear already staphylococci capable of producing such an enzyme [31]. The constitutive resistance to oxacillin is determined by the presence of the *mecA* gene, which is located on chromosome [32]. In a previous study investigated the prevalence of *Staphylococcus aureus* resistant to methicillin in an ICU, the value of these resistant microorganisms was 60.4%, which differs from the value found in this work that was 36.4% [5].



198
199
200

Fig. 3. Resistance percentage of strains of *Staphylococcus aureus* and *Staphylococcus spp.*



201

Fig. 4. Resistance percentage of strains of *Staphylococcus aureus* and *Staphylococcus spp.*

After analyzing the results of the test D, it was observed that the 07 bacterial strains that had erythromycin resistance, 04 strains were resistant to erythromycin and sensitive to clindamycin and negative induction test, which is interpreted as a mechanism of resistance for efflux (MSB). A strain showed resistance to erythromycin and clindamycin sensitivity to false positive induction test, which is interpreted as an amendment to ribosomal inductive resistance mechanism (MLSBi) and 02 strains were resistant to both erythromycin as clindamycin, and interpreted as modifying ribosomal with constitutive type resistance mechanism (MLSBc). Table 1 shows the results of resistance testing after induction.

The interpretive criteria for erythromycin predicting the same outcome for clarithromycin and azithromycin, may be used any of the three tests antibiotics [33]. However, in our study, the results between azithromycin and erythromycin proved to be discordant with 9.0 and 31.8% respectively, this fact may lead to treatment failure if these samples are isolated from infectious processes and suggest further study. A previous study resistotyping community strains of *S. aureus* strains isolated in João Pessoa and found partial resistance to macrolides of 14 and 15 members [34].

The macrolide resistance can be determined by *mrsA* gene, which mediates an efflux mechanism and confers resistance to macrolides and type B streptogramins, however, does not confer resistance to lincosamides (clindamycin and clarithromycin), or can occur by a change in ribosomes, which affects the activity of macrolides, lincosamides and type B streptogramins [35]. We can see that the resistance phenotype was most frequently found MSB with 18.2% of *S. aureus* strains isolated. This result is also consistent with the frequency found by previous studies, where the MSB phenotype was the most present, differing only in the incidence, since they showed a rate of 7.2% of *S. aureus* strains with type MSB resistance, as our study found a value more than twice [36].

In this study the frequency for constitutive resistance to clindamycin (MLSBc) was 28.6% among strains of *S. aureus* that showed erythromycin resistance. The resistance rate found in our study is close to the value found in the literature, which was assessed colonization by *Staphylococcus spp.* on surfaces of medical articles and nostrils and hands of professionals, finding the value of 20.4% of strains resistant to erythromycin with type MLSBc profile [37]. The MLSBi samples exhibit a high rate of spontaneous mutations for constitutive resistance which can hinder treatment and aggravate the problem of infections caused by *S. aureus* in hospitals and in the community setting [38]. In our study, the number of strains with MLSBi type resistance was lower compared to the values found for MLSBc, with a value of 14.3% among *S. aureus* strains resistant to erythromycin, ie 4.54 % of *S. aureus* isolates in the study. This value was close to the frequency of 5.2% obtained in previous studies [36].

Table 1. Values of resistance phenotypes observed after induction test

<i>Erythromycin</i>	<i>Clindamycin</i>	<i>Induction Test</i>	<i>Resistance Phenotype</i>	<i>Determinant gene</i>	<i>Percentage Resistance (%)</i>
R	S	-	MSB	<i>msrA</i>	57,1
R	S	+	MLSBi	<i>erm</i>	14,3
R	R	-	MLSBc	<i>erm</i>	28,6

243 **4. CONCLUSION**

244

245 The results of the present study demonstrate that the hospital environment is contaminated
246 with multidrug-resistant bacteria. Paramount importance periodic resistotyping with
247 antibiotics selected by competent bodies, whose resistances are important genetic and
248 epidemiological markers in order to distinguish new lines *Staphylococcus* spp., those
249 endemic, as well as a better understanding of the evolution of drug resistance in a unique
250 environment.

251

252 **CONSENT (WHERE EVER APPLICABLE)**

253

254 All the authors declare that no consent was obtained for this study.

255 **REFERENCES**

256

257 1. Cunha MLRS, Lopes CAM, Rugolo LMSS, Chalita LVAS. Clinical significance of
258 coagulase-negative staphylococci isolated from neonates. *J Pediatr*, v.78, n. 4, p. 279-288,
259 2002.

260 2. Cavalcanti SMM, França ER, Viela MA, Montenegro F, Cabral C, Medeiros ACR.
261 Comparative study on the prevalence of *Staphylococcus aureus* imported to intensive care
262 units of a university hospital, Pernambuco, Brazil. *Rer Bras Epidemiol*, v. 9(4), p. 436-446,
263 2006.

264 3. Blythe D, Keenlyside D, Dawson SJ, Galloway A. Environmental contamination due
265 to methicillin-resistant *Staphylococcus aureus* (MRSA). *J Hosp Infect.*, v. 38(1), p.67-69,
266 1998.

267 4. Oie S, Kamiya A. Contamination of environmental surfaces by methicillin-resistant
268 *Staphylococcus aureus* (MRSA). *Biomed Lett*, v. 57, p. 115-119, 1998.

269 5. Ferreira AM, Andrade D, Rigotti MA, Almeida MTG. Methicillin-resistant
270 *Staphylococcus aureus* on surfaces of an Intensive Care Unit. *Acta Paul Enferm*, v. 24(4), p.
271 453-458, 2011.

272 6. David CM. Infection In the Critically Ill Patient. *Medicine*, Ribeirão Preto 1998;
273 31:337-48.

274 7. Horn WA, Larson EL, Mcginley KJ, Leyden JJ. Microbial flora on the hands of health
275 care personnel: differences in composition and antibacterial resistance. *Infect Control Hosp*
276 *Epidemiol*, 1988; v.9, p. 189-193.

277 8. Behrman RE, Kliegman RM, Jenson HB. Nelson: treated pediatric. 17ª edição. Rio
278 de Janeiro: Elsevier; 2005.

279 9. Andrade D, Leopoldo VC, Haas VJ. Occurrence of Multi-Resistant Bacteria in the
280 Intensive Care unit of a Brazilian Hospital of Emergencies. *Rev Bras Ter Intensiva*, 2006;
281 v.18(1), p.27-33, 2006.

282 10. JrSousa FC, Nunes EWF, Nascimento ED, Oliveira SM, Melo MCN, Fernandes
283 MJBC. Prevalence of methicillin-resistant *Staphylococcus* spp isolated in a teaching

- 284 maternity hospital in the city of Natal, State of Rio Grande do Norte. Rev. Soc. Bras. Med.
285 Trop, 2009; v.42(2), p. 179-182.
- 286 11. Mundim GJ, Dezena RA, Oliveira ACS, Silva PR, Cardoso M, Pereira GA, Morais
287 CA, Terra APS. Evaluation of presence of Staphylococcus aureus on the beds of Hospital
288 Escola's Intensive Care Unit, concerning the position on the mattress, before and after
289 cleaning. Rev Soc Bras Med Trop, 2003; v. 36(6), p. 685-688.
- 290 12. Zanconato RV, Pereira WKV, Abegg MA. Microbiological condition of hospital
291 mattresses before and after disinfection. Hospital Practice, 2007 July/Aug; 52(9):68-72.
- 292 13. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of
293 the resistance elements and their clinical implications. Clin Infect Dis, 2002; 34: 482-92.
- 294 14. Weisblum B. Erythromycin resistance by ribosome modification. Antimicrob. Agents
295 Chemother., 1995; 39, 577-585.
- 296 15. Oplustil CP, Zoccoli ZM, Tobouti NR. Basic Procedures in Clinical Microbiology. 2.
297 ed. São Paulo: Sarvier, 2004.
- 298 16. Turng J. Detection and Interpretation of Macrolid- Lincosamide- Streptogramin
299 Resistance among staphylococcus with Phoenix Automated Microbiology System and
300 BDXpert System, As presented at the 15th European Congress of Clinical Microbiology and
301 Infectious Disease (ECCMID), Copenhagen, 2005.
- 302 17. Allignet J, Loncle V, Solh N. Sequence of a staphylococcal plasmid gene, vga,
303 encoding a putative ATP-binding protein involved in resistance to virginianmycin A-like
304 antibiotics. Gene, 1992; 117, 45-55.
- 305 18. Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Kohrer K, Verhoef J, Fluit Ad C.
306 Prevalence of macrolideresistance genes in Staphylococcus aureus and Enterococcus
307 faecium isolates from 24 European university hospitals. J Antimicrob Chemother, 2000; 45,
308 891-894.
- 309 19. Drinkovic D, Fuller ER, Shore KP et al. Clindamycin treatment of Staphylococcus
310 aureus expressing inducible clindamycin resistance. J Antimicrob Chemother, 2001; 48(2):
311 315-16.
- 312 20. Frank AL, Marcinak JF, Mangat PD et al. Clindamycin treatment of methicillin-
313 resistant Staphylococcus aureus infections in children. Pediatr Infect Dis J, 2002; 21: 530-
314 34.
- 315 21. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-
316 resistant Staphylococcus aureus expressing inducible clinda-mycin resistance in vitro. Clin
317 Infect Dis, 2003; 37(9): 1257-60.
- 318 22. Levin TP, Suh B, Axelrod P et al. Potential clindamycin resistance in clindamycin-
319 susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure.
320 Antimicrob Agents Chemother, 2005; 49(3): 1222-24.
- 321 23. Fiebelkorn KR, Crawford SA, Mcelmeel ML, Jorgensen JH. Practical disk diffusion
322 method for detection of inducible clindamycin resistance in Staphylococcus aureus and
323 coagulase-negative staphylococci. J Clin Microbiol, 2003; 41: 4740-44.

- 324 24. Rossi F, Andreazzi DB. Bacterial resistance: playing the antibiogram. 1th ed. São
325 Paulo, Atheneu, 2005, p. 118.
- 326 25. Ferreira AM, Andrade D, Almeida MTG, Cunha KC, Rigotti MA. Egg crater
327 mattresses: a deposit of methicillin-resistant *Staphylococcus aureus*?. Rev Esc Enferm USP,
328 v.45(1), p.161-166, 2011.
- 329 26. Xavier MS, Ueno M. Bacterial contamination of stethoscopes in pediatric units at a
330 university hospital. Rev Bras Med Trop, 2009; v.42(2), p.217-218.
- 331 27. Araujo BAC, Oliveira AL, Filho LS. *Staphylococcus aureus* multi-resistant strains
332 isolation from stethoscopes used in nosocomial environment. Rev. bras. anal. clin, v. 32(4),
333 p.285-288, 2000.
- 334 28. Oliveira AC, Damasceno QS. Surfaces of the hospital environment as possible
335 deposits of resistant bacteria: a review. Rev Esc Enferm USP, 2009; v.44(4), p.1118-1123.
- 336 29. Tavares W. Problem gram-positive bacteria: resistance in staphylococci,
337 enterococci, and pneumococci to antimicrobial drugs. Rev Bras Med Trop, 2000; v.33(3),
338 p.281-301.
- 339 30. Martins SCS, Martins CM, Albuquerque LMB, Fonteles TV, Rego SL, Junior GSF.
340 Profile resistance of positive coagulase *Staphylococcus* Strains isolated from food handlers.
341 Bol Centro Pesqui Process Aliment, 2009; v. 27, n. 1.
- 342 31. Mussi ESRH, Medeiros FA, Santos AM, Rangel RCS, Medeiros AAN. Incidence of
343 *Staphylococcus aureus* resistant to oxacillin at hospital/Macapá and evaluation of
344 susceptibility to extract of *Licania macrophylla* Bent. Cie Equat, v.2, nº 1, 2012.
- 345 32. Colli VC, Pizzolitto AC, Raddi MSG. Identification of drug resistance in
346 *Staphylococcus aureus*: a challenge?. Rev Ciên Farm Básica Apl, 2009; v. 30, p. 99-102.
- 347 33. Clsi editor (2012), Clinical and Laboratory Standards Insitute. Performance
348 Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement
349 M100-S22. CLSI, Wayne, PA, USA.
- 350 34. Lima ZN. Resistotyping community human strains of *Staphylococcus aureus*
351 isolated in the state of Paraíba. Dissertation (Master in Genetics). Center of Exact Sciences
352 and of the Nature by Federal University of Paraíba, João Pessoa, 2002, 77 f.
- 353 35. Mendes, CMF, Oplustil CP, Zoccoli CM, Sinto SI. Clinical Microbiology: 156
354 questions and answers. 1th ed. São Paulo: Sarvier, 2005, p.323.
- 355 36. Merino Díaz L, Torres Sánchez MJ, Aznar Martín J. Prevalence and mechanisms of
356 erythromycin and clindamycin resistance in clinical isolates of β -haemolytic streptococci of
357 Lancefield groups A, B, C and G in Seville, Spain. Clin Microbiol Infec, 2008; 14: 85–87. doi:
358 10.1111/j.1469-0691.2007.01881.x.
- 359 37. Rodrigues, AMSB. Occurrence and Characterization Pathogenic Species of
360 *Staphylococcus* genus in Articles Medical and Health Practitioners in two Basic Health Units
361 in the city of Rio de Janeiro, from 2009 to 2011, Brazil. Dissertation (Professional Master).
362 Oswaldo Cruz Foundation, National Institute of Quality Control in Health, Graduate Program
363 in Health Surveillance, Rio de Janeiro, 2011, 103 f.

364 38. Patel M, Waites KB, Moser SA, Cloud GA, Hoesley CJ. Prevalence of inducible
365 clindamycin resistance among community and hospital associated *Staphylococcus aureus*
366 isolates. *J Clin Microbiol* 2006;44:2481-4.